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Increased LH and FSH Secretion After Cranial Irradiation in Boys

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The effect of high-dose cranial- and craniospinal irradiation and chemotherapy on the gonadotropin-sex steroid axis was studied during different stages of puberty by measuring pulsatile secretion of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone. The patients were thirteen boys who had been treated for malignant brain tumor residing well away from the hypothalamo-pituitary region. The median time to follow-up was 9 (1–16) years. The onset of puberty was early in the patients, median 10.5 years, compared to the average age for Swedish boys, which is at median 12.4 years. There was, before puberty, no significant difference in LH and FSH secretion between patients and a control group of normal boys. In early, mid- and late stages of puberty, however, LH and FSH secretion was increased in the patients overall, whereas testosterone secretion was maintained within the normal range in spite of signs of gonadotoxocity with small testicular volumes. These results indicate that the vulnerable parts of the gonadotropin releasing hormone (GnRH)-gonadotropin (LH, FSH)-gonadal axis are the regulatory system that determines the timing of pubertal induction and the gonads. The GnRH-LH, FSH-releasing neurons appear relatively resistant to cranial irradiation as they are able to respond with supranormal LH and FSH levels for long periods of time after treatment. Med Ped Oncol 29: 280–287, 1997. © 1997 Wiley-Liss, Inc.

Key words: LH; FSH; testosterone; puberty; brain tumor; cranial irradiation; chemotherapy

INTRODUCTION

Children with malignant brain tumors, residing well away from the hypothalamo-pituitary axis, (HPA axis), are usually treated with high-dose radiotherapy to the tumor area. Whole brain and craniospinal radiation as well as chemotherapy may also be part of the therapeutic arsenal. In this context, radiation therapy is known to cause growth hormone (GH) deficiency with time owing to a central damage to the hypothalamus. Hypothyroidism, on the other hand, occurs more often from a peripheral damage as scatter from the craniospinal radiation also hits this gland [1]. However, the gonadotropin releasing hormone (GnRH)-(LH, FSH) axis has not been systematically studied following cranial irradiation in children.

Tumors residing in the HPA axis often cause immediate and total gonadotropin (Gn) deficiency as does surgery in this region [2]. After radiation therapy, the clinical symptoms of damage take longer time to develop depending on the dose administered. There may be a disturbance in the onset and in the progression of puberty and later in life, impairment of fertility. Precocious and early onset of puberty have been reported after total doses of radiotherapy as low as 18 Gy [2,3], whereas delayed onset of puberty or pubertal insufficiency may occur after very high doses of radiotherapy given directly to the hypothalamic region [4]. In addition to radiotherapy, children with brain tumors often receive chemotherapy with alkylating agents. This treatment is known to have detrimental effects on the germ cells, which make up for the major part of the testicular volume, whereas the Leydig cells which produce testosterone are much less affected by chemotherapy. Furthermore, the inadvertent scatter radiation to the gonads from spinal radiation needs to be considered even if the testes are shielded. A total dose of only 6 Gy could potentially cause azoospermia.

Leydig cell dysfunction is seen after total doses >20 Gy. As a consequence of this combination treatment, testicular volumes may be inappropriately small and soft in long-term survivors compared to normal boys, but testosterone levels usually remain within normal limits [5,6].

In normal children, we have recently shown that irregular LH and FSH pulses occur already before the onset of puberty [7], in agreement with findings by Wu et al. [8]. Other investigators have published contradictory

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TABLE I. Clinical Data at Tumor Diagnosis and at First and Second LH-FSH Investigation

Patient	Age at tumor diagnosis (y)	RT[a] (Gy) chemo ± spinal RS (s)	First investigation			Second investigation			Age at onset of puberty (y)
			Age (y)	Puberty stage	Testes (ml)	Age (y)	Puberty stage	Testes (ml)	
1	2	40 (+) (s)	5	pre 1	1	9	pre 2	2	10
2	3	40 (−)	4	pre 1	1	14	late	25[c]	10
3	6	56 (+) (s)	10	pre 1	1	–	–	–	†
4	10	56 (+)	11	pre 2	2	13	early	6	12
5	1	40 (+) (s)	10	early	3	–	–	–	9
6	12	40 (−)	13	early	6	–	–	–	b.d.[b]
7	6	56 (+)	17	early	4[c]	–	–	–	11
8	14	56 (+)	15	mid	15	23	late	12	b.d.
9	5	40 (+) (s)	15	mid	15	–	–	–	11
10	6	40 (+) (s)	15	late	3	–	–	–	10
11	11	40 (+) (s)	19	late	25[c]	23	late	15	13
12	4	50 (+)	20	late	25[c]	–	–	–	11
13	17	56 (+) (s)	23	late	15	–	–	–	b.d.

[a]RT, radiotherapy.
[b]b.d., before tumor diagnosis.
[c]On GH substitution therapy.
†Dead.

results on this issue possibly owing to differences in methodology [9,10]. In normal children, we also found a clear covariation of LH and FSH secretion indicating a synchronization of LH and FSH secretion.

In the present study, we used an ultrasensitive time-resolved immunofluorometric assay (tr-IFMA) which is about 100 times more sensitive than RIA radioimmunoassays and 5–10 times more sensitive than IFMA methods [11,12] in detecting LH and FSH pulses.

Exploiting the greater sensitivity of the tr-IFMA assay on LH and FSH, this partly longitudinal descriptive study aimed to investigate the physiological secretion of LH and FSH, i.e., the existence of LH and FSH pulses, their amplitude and diurnal variation from prepuberty to late puberty, in a group of boys who had received combination treatment with cranial/craniospinal irradiation and chemotherapy for a brain tumor.

PATIENTS AND METHODS

Patients

Thirteen boys who had previously been treated for a brain tumor well away from the parasellar region (medulloblastoma posterior fossa, 7; ependymoma posterior fossa, 1; astrocytoma hemisphere, 5) were investigated. Treatment was surgery, cranial ± craniospinal radiotherapy and in most cases chemotherapy. At the time of tumor diagnosis, the patients were between 1 and 17 years old (Table I). The total radiation doses delivered to the HPA region were between 40 and 56 Gy. Spinal radiation was given to seven patients (Table I) to a median dose of 32 Gy (range 20–32) specified at a median depth of 2.5 cm. Chemotherapy included Vincristine and the two alkylating agents CCNU and Procarbazine, which were given in repeated cycles for up to one year. Eighteen investigations of LH, FSH and testosterone secretion were performed between 1 and 16 years, median 9 years, after the tumor diagnosis, at which time all patients were in good health and all treatment had been discontinued. By the time of the first investigation, five patients were prepubertal and eight were already in different stages of puberty. In five children, studies were performed twice but during different stages of puberty.

In normal children, the onset of puberty is considered to have occurred when testicular volumes reach 4 ml. In our control group of normal children, however, we saw that with testicular volumes of 3 ml, gonadotrophin levels were clearly higher than with testicular volumes 1–2 ml. Our control group (see below) was therefore divided into prepubertal stage 1 (testicular volumes of 1–2 ml) and prepubertal stage 2 (testicular volume 3 ml) [7]. In the patient group, this subdivision was difficult since testicular volumes often remain smaller and softer after gonadotoxic radio- and chemotherapy compared to what is normal for each pubertal stage. Therefore, testicular volumes cannot be used for adequate pubertal staging. To make it possible to graphically demonstrate the development of the LH-FSH profiles in the patients compared to the control group, we subdivided the prepubertal patients as follows: the youngest patients with a testicular volume of 1 ml were called pre 1; the somewhat older patients who had their profile done within a year before onset of puberty and in whom the testicular volumes showed some growth (2 ml) were called prepubertal stage 2.

Control Group

The control group was made up of twelve healthy boys aged between 8.7 and 18.2 years, investigated re-

peatedly before puberty and during the different stages of puberty over a period of 2.5–9.5 years. This added up to a total of 54 profiles of LH, FSH and testosterone levels. Data from subjects with more than one observation per pubertal stage were averaged in the graphs and for statistical analyses. Thus, five profiles were performed on boys in stage pre-1, six in stage pre-2, eleven in early puberty, eight in mid-puberty, and nine in late puberty [13].

Study Protocol

The study was conducted at the Children's Hospital Göteborg. Both patients and control group followed the same study protocol. The boys stayed at the hospital at least two days. A heparinized needle (Carmeda AB, Stockholm, Sweden) was inserted the first evening or morning. In the morning at 08.00–09.00 blood specimen collection began. A constant withdrawal pump (Swemed, Göteborg, Sweden) with a nonthrombogenic catheter (Carmeda) was used [14]. The rate of withdrawal was 0.5–2 ml/h, and volume of the testing system 0.1–0.2 ml. The heparinized tubes were changed every 20 min for 24 h, thus giving 72 samples. The heparinized tubes of blood were stored at room temperature and centrifuged within 24 h. After centrifugation, the plasma samples were frozen and stored until assayed for LH and FSH.

Pubertal stage was estimated by genital size according to Tanner [15] and by pubic hair (PH). Testicular volumes were determined by comparison with Prader's orchidometer. Informed consent was obtained from each child and his parents. The protocol was approved by the Ethical Committee of the Medical Faculty, University of Göteborg.

Hormone Determinations

Plasma LH and FSH concentrations were measured by time-resolved IFMAs, using reagents from Wallac (Kabi Pharmacia, Turku, Finland), as previously described [7,11]. The LH standards were calibrated against WHO International Reference Preparation 68/40, and FSH standards against the Second International Reference Preparation of pituitary FSH/LH (78/549). All measurements were made in duplicate. The assay sensitivity for LH was 0.019 IU/L, and that for FSH was 0.014 IU/L, as defined by the mean ± 2 S.D. of 12 replicates of a zero sample. The intra-assay coefficient of variation (CV) for FSH ranged from 2.1% (at 64 IU/L) to 8.5% (at 0.1 IU/L), and for LH from 3.1% (at 50 IU/L) to 13.9% (at 0.1 IU/L). The interassay CVs ranged from 3.6% to 4.1% for FSH and from 5.4% to 5.6% for LH at concentrations around 20 and 5 IU/L, respectively.

Testosterone concentrations were determined by RIA using coated tube technology (Spectria) from Orion Diagnostics (Espoo, Finland). The volume of serum used was 50 μL instead of 25 μL, in order to increase the sensitivity of the kit; otherwise, the RIA was conducted according to the manufacturer's instructions. The detection limit was 30 pmol/L. The intra-assay coefficient of variation was 31% for 0.19 nmol/L and below 7.4% for 0.92 nmol/L and higher concentrations.

Pulse analyses for LH and FSH were performed using a computerized pulse analysis program, Pulsar [16]. The program identifies secretory peaks by height and duration from a smoothed baseline, using the assay S.D. as a scale factor. The cut-off parameters G 1–5 of the Pulsar program were set to 2.5, 1.5, 1, 0.75, and 0.5 times the intra-assay S.D. as criteria for accepting peaks 1, 2, 3, 4, and 5 points wide, respectively, and peak-splitting parameter set to 1.5. With these settings, the program did not detect any peaks when 55 consecutive samples from each of two different plasma pools was assayed. Missing values comprised less than 3% of the total samples and were left blank.

Cross-correlation was used to compare interrelationships between LH and FSH. This is a technique for assessing the time relationship between two data series. The data are made stationary and progressively moved at intervals corresponding to the sampling interval. The data are regressed with each move and a correlation coefficient generated for each point between LH and FSH.

Statistics

Data are given as mean ± S.E.M. Differences between the groups were assessed by Mann-Whitneys U-test. A P-value < 0.05 was considered significant. Distribution of peak amplitudes was compared with χ^2-analysis.

RESULTS

LH

In prepubertal boys, low but measurable LH levels (mean 0.54 ± 0.23 U/L) were seen. Irregular peaks up to 4 IU/L over the 24-h period occurred but the levels did not differ from the control group (Fig. 1).

In early puberty, a day-night rhythmicity was observed (Fig. 1), and the mean level increased to 4.52 ± 0.74 U/L. The number of peaks = the pulse frequency was not significantly different in patients and controls (Fig. 2, top panel). In the two boys investigated in mid-puberty, mean levels of LH were 3.6 and 7.6 U/L.

In early, mid- and late puberty, mean levels of LH were markedly elevated compared to the control group. This was due to a gradual increase in the basal secretion (Fig. 2, middle panel) as well as in the peak amplitude (Fig. 3). The highest LH levels were seen in a boy (patient 10) with the smallest testicular volume. The lowest LH levels were seen in a boy (patient 8) who received a very high dose radiotherapy to the central part of the brain although not directly to the parasellar region.

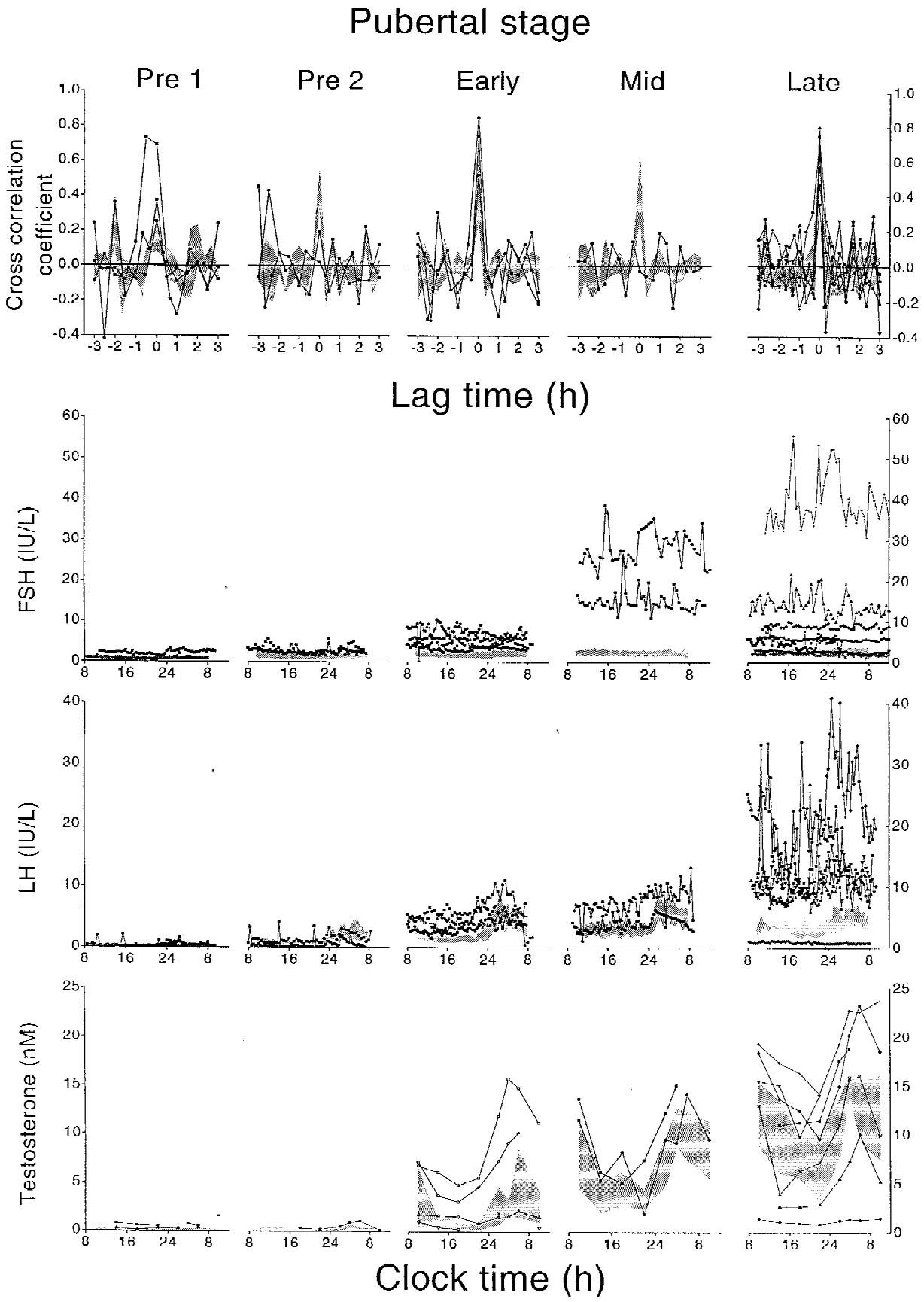

Fig. 1. Boys treated for brain tumor compared to healthy control boys (shaded areas) for hormone levels of LH and FSH (IU/L) and testosterone (nM) over 24 hours at different stages of puberty. Cross-correlation between LH and FSH secretion (top). Data on the control group is given as the values between the 25th and the 75th percentile.

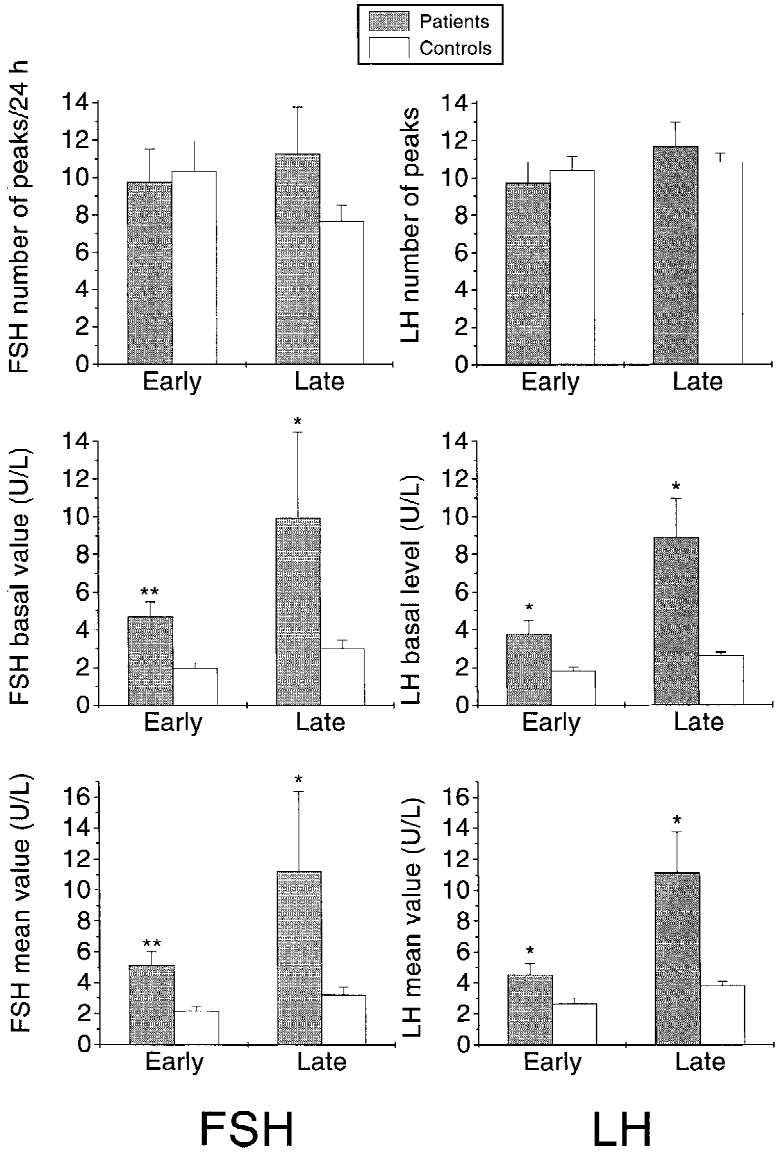

Fig. 2. Analysis of LH and FSH secretion in boys treated for brain tumor compared to healthy control boys for peak number, basal and mean value during 24 hours. In early and late puberty, 4 and 7 patient profiles, respectively, were compared to 11 and 9 profiles in the controls. Each bar represents the mean value ± S.E.M. for all children in that pubertal stage. *$P < 0.05$ and **$P < 0.01$ vs. corresponding control group with Mann-Whitney U-test.

FSH

In prepubertal boys the mean FSH level was 1.84 ± 0.45 U/L. Irregular peaks were seen in some patients.

In early puberty, the mean FSH level increased to 5.15 ± 0.89 U/L. In mid- and late puberty, markedly elevated FSH levels became more pronounced. This was due to a raised basal secretion compared to the control group (Fig. 2) as well as a marked increase in peak amplitude with frequent peaks reaching peak maximal levels above 20–25 IU/L. The highest FSH levels were seen in the same boy (patient 10) who had very high LH levels and the lowest FSH levels in the boy (patient 8) who had the lowest LH levels. The number of peaks = the peak frequency did not differ from the control group in the early and late group (Fig. 2).

Cross-Correlation

In early puberty and late puberty (study patients too few in mid-puberty), there was a clear cross-correlation for LH and FSH, indicating a synchronized release of these hormones (Fig. 1).

Testosterone

The levels of testosterone were essentially normal before puberty and in early stages of puberty. In late puberty, two boys, patients 8 and 10, had very low testos-

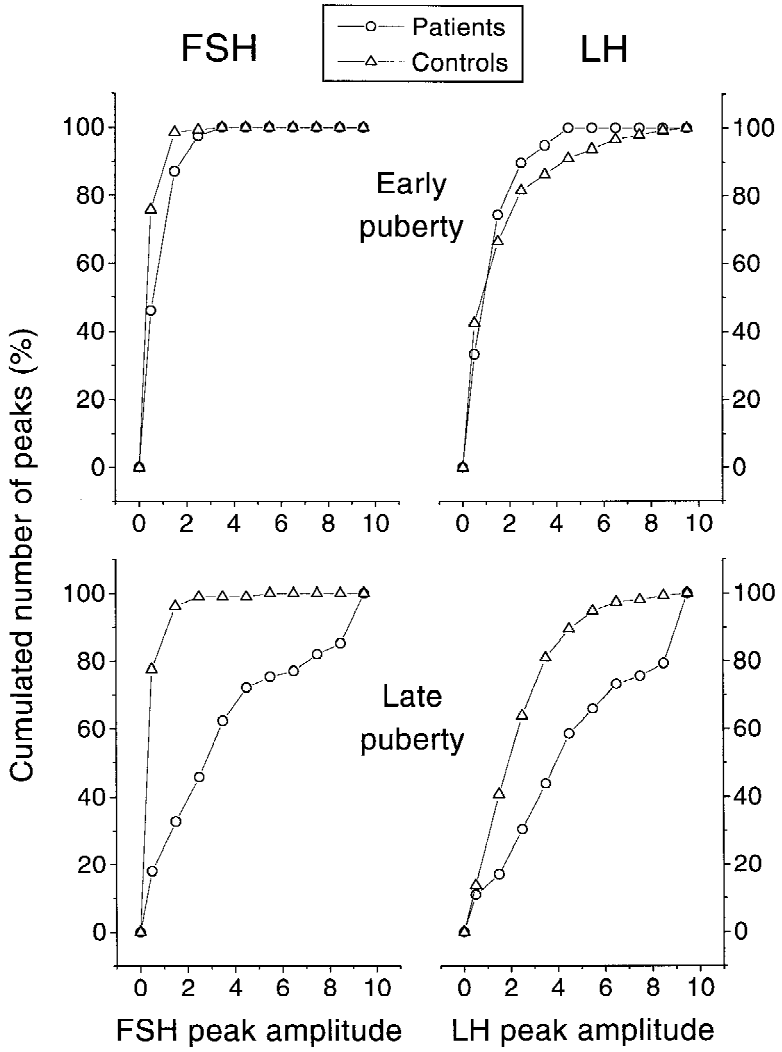

Fig. 3. Analysis of LH and FSH peak amplitude in patients and controls in early and late puberty. In early and late puberty, 4 and 7 patient profiles, respectively, were compared to 11 and 9 profiles in the controls. A shift to the right in the cumulative frequency curve indicates a higher percentage of peaks with high amplitude. The difference between patients and controls was significant ($P < 0.001$, χ^2-analysis) for both LH and FSH in late puberty and for LH in early puberty ($P < 0.01$).

terone levels. Patient 8 had very low gonadotrophins after receiving a high-dose RT to central parts of the brain. Patient 10 had small testes and very high gonadotropins. The rest of the boys had testosterone levels within the normal range of our control boys and a clear day-night variation as in normal boys.

Onset of Puberty

In boys irradiated before 11 years of age, onset of puberty was a median 10.5 years, which is significantly early compared to the Swedish reference data which have estimated the average age at onset of puberty for boys to be 12.4 years (Table I) [17].

DISCUSSION

The major result of this descriptive study is that increased levels of LH and FSH are present together with normal testosterone levels many years after multimodality treatment for a brain tumor residing well away from the hypothalamic-pituitary region. Thus, the GnRH-LH, FSH-testosterone axis seems to be relatively resistant to high-dose cranial irradiation at least within a 10-year period from treatment. This is in contrast to the gradually developing deficiency of growth hormone that these patients experienced within a couple of years after their radiation treatment. Although none of the boys had their tumor in the hypothalamic region, the radiation dose to this region was high, in several patients, 56 Gy. The number of patients was, however, too small to allow for a more detailed analysis regarding time after therapy and radiation dose in relation to LH, FSH and testosterone secretion.

Although normal or even supernormal levels of LH and FSH were maintained for long time after radiotherapy to the hypothalamic-pituitary region, the control

of the onset of puberty was affected as shown by the disturbed timing of pubertal onset in this study. This finding confirms previous studies [2,3]. Ogilvy-Stuart et al. [3] analyzed the relation between age at cranial irradiation and pubertal onset in a larger group of patients and have proposed a model that predicts the time for onset of puberty to 9.21 years + 0.29 years for every year of age at cranial irradiation in boys [3]. Five of our eight patients who were followed into puberty fit this model well, whereas two had an earlier and one a later onset of puberty than the model predicted.

The great sensitivity of the present LH and FSH assays enabled us to detect irregular LH and FSH pulses already before puberty. This pattern was, however, not different from what was observed in the control group of normal boys [13]. Therefore, we could not detect any abnormalities, i.e., ''warning signs,'' before the clinical onset of puberty in the patients. Furthermore, at the earliest pubertal stage, an increment in LH secretion was detected in the patients, especially during the night, leading to a diurnal variation. These results were also in concordance with our findings in normal boys, where we could observe an increase in nocturnal LH pulse frequency and amplitude during the clinical transition from prepuberty to early puberty.

Each LH pulse putatively results from a GnRH pulse, as has been shown in some experimental models [18,19]. Our results suggest that pituitary LH secretion is highly sensitive to endogenous GnRH even before puberty [13] and that this is true also after cranial irradiation to this region. This is in agreement with previous reports indicating that the pituitary is relatively resistant to radiotherapy compared to the hypothalamus.

In later stages of puberty, however, there were striking differences between the patients and the control group as the patients demonstrated LH and FSH far in excess over controls. This was due both to raised baseline levels and to increased peak amplitudes of secretion of LH and FSH. Despite these changes in amounts of hormones, the number of peaks and the synchronization of secretion was maintained. This could indicate that the central pulse-regulating mechanism is intact in these patients, but the negative feedback mechanism from the testes is damaged which stimulates increased basal levels of LH and FSH.

In our prepubertal patients, plasma LH and FSH levels were in the normal low range and FSH levels were clearly higher than LH levels in normal children. In normal children, this pattern is reversed in late puberty. However, in keeping with the well-known greater sensitivity to cytotoxic drugs of the germ cells, responding to FSH, compared to the Leydig cells, responding to LH, we observed higher FSH than LH levels in most but not all children in late puberty. The only child who had not received chemotherapy showed the normal LH > FSH but both levels were raised. Again, the most obvious finding in puberty was the very high levels of both these hormones. One could speculate if the rather long follow-up time in this study has allowed also for the Leydig cells to become affected (from chemotherapy and spinal scatter radiation) to such an extent that by lack of negative feedback, LH levels almost reaches those of FSH. (The patient with the highest LH levels in our study also had the smallest testicular volume and the lowest testosterone production.) Therefore, it may be that the normal testosterone levels that we now measured will decrease with even longer follow-up. Furthermore, cytotoxic testicular damage may also affect other feedback factors such as inhibin, activin and follistatin which are probably very important in modulating the gonadotropin secretion, perhaps more important than the GnRH pulse frequency per se as shown by Bridges et al. [20].

The GnRH-FSH/LH-gonadal axis is fundamental for sexual maturation and fertility, making the body ready for pubertal onset at any point if the inhibiting mechansims are disturbed in some way. There are a also a number of feedback interactions within this system with the purpose of ensuing normal function and fertility. Consequently, the damage afflicted by high dose cranial irradiation is not a gradual decline in LH and FSH levels similar to that seen for GH. Instead, an early onset of puberty occurs and in most cases a normal progression through puberty follows with testosterone levels within the normal range in spite of small testes. In later stages of puberty, probably a loss of feedback mechanisms from gonadal damage will result in very high levels of LH and FSH compared to normal children. Whether the central or the peripheral damage afflicted by multimodality treatment eventually leads to lowered or decreasing levels of gonadotrophins and testosterone remains to be seen.

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REFERENCES

1. Albertsson-Wikland K, Lannering B, Marky I, Mellander L, Wannholt U: A longitudinal study on growth and spontaneous growth hormone (GH) secretion in children with irradiated brain tumours. Acta Paediatr Scand 76:966–973, 1987.
2. Brauner R, Rappaport R: Precocious puberty secondary to cranial irradiation for tumours distant from the hypothalamo-pituitary area. Hormone Res 22:78–82, 1985.
3. Ogilvy-Stuart AL, Clayton PE, Shalet SM: Cranial irradiation and early puberty. J Clin Endocrinol Metab 6:1282–1285, 1994.
4. Rappaport R, Brauner R, Czernikow P: Effect of hypothalamic

and pituitary irradiation on pubertal development in children with cranial tumors. J Clin Endocrinol Metab 54:1164–1168, 1982.
5. Livesey EA, Brook CG: Gonadal dysfunction after treatment of intracranial tumours. Arch Dis Child 63:495–500, 1988.
6. Ahmed S, Shalet S, Campbell R, Deakin O: Primary gonadal damage following treatment of brain tumors in childhood. J Pediatr 103:562–565, 1983.
7. Dunkel L, Alfthan H, Stenman U-H, Selstam G, Rosberg S, Albertsson-Wikland K: Developmental changes in 24-hour profile of luteinizing hormone and follicle-stimulating hormone from prepuberty to midstadges of puberty in boys. J Clin Endocrinol Metab 74:890–897, 1992.
8. Wu FCW, Butler GE, Kelnar CJH, Sellar RE: Patterns of pulsatile luteinizing hormone secretion before and during onset of puberty in boys: A study using an immunoradiometric assay. J Clin Endocrinol Metab 70:629–637, 1990.
9. Garibaldi LR, Picco P, Magier S, Chevli R, Aceto T Jr: Serum luteinizing hormone concentration, as measured by a sensitive immunoradiometric assay, in children with normal, precocious or delayed pubertal development. J Clin Endocrinol Metab 72:888–898, 1991.
10. Oerter KE, Uriarte MM, Rose SR, Barnes KM, Cutler GB Jr: Gonadotropin secretory dynamics during puberty in normal girls and boys. J Clin Endocrinol Metab 71:1251–1258, 1990.
11. Dunkel L, Alfthan H, Stenman U-H, Perheentupa J: Gonadal control of pulsatile secretion of luteinizing hormone and follicle-stimulating hormone evaluated by ultrasensitive time-resolved immunofluorometric assays. J Clin Endocrinol Metab 70:107–114, 1990.
12. Wu FCW, Butler GE, Kelnar CJH, Stirling HF, Huhtaniemi I: Patterns of pulsatile luteinizing hormone and follicle-stimulating hormone secretion in prepubertal (midchildhood) boys and girls and patients with idiophatic hypogonadotropic hypogonadism (Kallmann's syndrome): A study using an ultrasensitive time-resolved immunofluorometric assay. J Clin Endocrinol Metab 72:1229–1237, 1991.
13. Albertsson-Wikland K, Rosberg S, Lannering B, Dunkel L, Selstam G, Norjavaara E: 24-hour profiles of luteinizing hormone, follicle-stimulating hormone testosterone and oestradiol levels: A longitudinal study throughout puberty in healthy boys. J Clin Endocrinol Metab 1997, in press.
14. Albertsson-Wikland K, Rosberg S, Libre E, Lundgren L-O, Groth T: Growth hormone secretory rates in children as estimated by deconvolution analyses of 24 h plasma concentration profiles. Am J Physiol 257 (Endocrinol Metab 20): E809–E814, 1989.
15. Tanner JM, Whitehouse RH: Clinical longitudinal standards for height, weight, height velocity, weight velocity and stages of puberty. Arch Dis Child 51:170–179, 1976.
16. Merriam GR, Wachter KW: Algorithms for the study of episodic hormone secretion. Am J Physiol 243:E310–E318, 1982.
17. Karlberg P, Taranger J, Engström I, Lichtenstein H, Svennberg-Redgren I: The somatic development of children in a Swedish urban community. Acta Paediatr Scan (Suppl) 258:1–148, 1976.
18. Levine KE, Pau K-Y, Ramirez VD, Jackson GL: Simultaneous measurement of LHRH and LH release in unanesthetized, ovariectomized sheep. Endocrinology 111:1449–1455, 1982.
19. Clarke IJ, Cummins JT: The temporal relationship between GnRH and LH secretion in the ovariectomized ewes. Endocrinology 111:1737–1739, 1982.
20. Bridges NA, Hindmarsh PC, Matthews DR, Brook CGD: The effect on changing gonadotropin-releasing hormone pulse frequency on puberty. J Clin Endocrinol Metab 3:841–847, 1994.